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COMPARATIVE ANALYSIS OF GENETIC DIVERSITY AMONG 24 GROUNDNUT GENOTYPES USING RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD) AND INTER SIMPLE SEQUENCE REPEAT (ISSR) MARKER

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ABSTRACT

Random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) were assayed to determine the genetic diversity of 24 genotypes of groundnut grown in Udaipur region of Rajasthan. RAPD and ISSR analysis showed high percent of polymorphism 90.23 and 85.71 respectively. The average PIC and Jaccard's similarity coefficient values for RAPD and ISSR was 0.216 ranging from 0.0319 to 0.449; 0.07 to 0.37 with an average of 0.300 and 0.63-0.91 with an average of 0.79; 0.54-0.90 with an average of 0.72 respectively. Based on the dendrogram generated by UPGMA method and PCA, most of the genotypes could be divided into four main clusters for RAPD and five clusters for ISSR.

KEYWORDS: RAPD, ISSR, UPGMA & PCA

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Original Article

INTRODUCTION

Groundnut is one of the most important crop species in the World and has been subject to considerable genetic studies. It is self-fertilizing species with a large genome. The identification of the varieties has become increasingly important to the documentation of genetic resources and for the protection of these genotypes for breeding programs. The cultivated peanut or groundnut (*Arachis hypogaea L*) originated in South America and is now grown throughout the tropical and warm temperate regions of the world. Groundnut is an allotetraploid (2n = 4x = 40), belonging to the genus *Arachis* that includes 70 species. Cultivated groundnut is divided into two subspecies: *Arachis hypogaea spp. Hypogaea* and *Arachis hypogaeassp. Fastigiata* Waldr., which are further classified into four botanical varieties (Kumazawa and Nishimura, 1953; Kraovickas and Gregory, 1994). Therefore, a significant scope to improve groundnut productivity in the region by exploiting germplasm diversity. Considerable variation has been recorded for morphological, physiological, and agronomic traits in groundnut crop. Groundnut varieties classified into four species market types (Spanish, Valencia, Virginia, and Southeast Runner). It is very difficult to classify accessions solely according to their morphological traits which can be affected by environmental conditions. DNA-based technologies are the most reliable tools for assessing genetic variability because they are not influenced by plant growing conditions. Different molecular markers have been used to study polymorphism in groundnut, random amplified polymorphic DNA (RAPD), and inter simple sequence repeats (ISSR) (Dwivedi et al., 2001; Raina et al., 2001; Herselman, 2003). The main objective of the present study was to analyze the level of molecular variation and genetic relatedness among 24 genotypes of

groundnut by using 19 RAPD and 18 ISSR markers.

MATERIALS AND METHODS

Genetic Materials

The experimental material comprised of 24 groundnut genotypes, including three checks, namely UG-5 (Pratap Raj Mungphali), PM-2 and GG-7. The 24 genotypes were procured from Department of Plant Breeding and Genetics, RCA Udaipur. Details of the source and pedigree of material used are given in Table 1.

DNA Extraction

DNA was extracted based on the style trimethyl ammonium bromide (CTAB) method as described by (Doyle and Doyle 1990). The quality and concentration of DNA were assessed spectrophotometrically and also by agarose gel electrophoresis (AGE) using 0.8 percent agrees with known concentration of uncut lambda DNA. A total of 19 RAPD and 18 ISSR primers was used for diversity analysis.

RAPD and ISSR Analysis

In brief, reproducible and clear banding patterns were obtained with a reaction mixture of 20 µl contain in 50 ng of template DNA, 2 µl of 10X *Taq* DNA polymerase buffer, 1.5 mm $MgCl_2$, 200 µM of each dNTP, 0.30 µM of primer and 1 U of *Taq* DNA polymerase. The annealing temperature was 37 C found to be optimum for proper annealing. For ISSR primers the annealing temperature varied from 28.9^0C to 59^0C for PCR amplification. Therefore, the thermo cycle was programmed for an initial denaturation step of 4 min. At 94^0C, followed by 40 and 35 cycles of denaturation (94^0C, 45 Sec.) for RAPD and ISSR; was optimized for annealing (37^0C, 1 min. For RAPD, 28.9^0C to 59^0C, 1 min. For ISSR primer) and extension (72^0C, 2 min. For RAPD and ISSR) followed by a final extension of 72^0C for 10 min. Temperature of 4^0C at the end that resulted in clear and reproducible bands. Earlier, almost similar PCR conditions were recorded for estimation of genetic variability in *A. hypogaea* L. Genotypes. Following the PCR amplification, the PCR products of both RAPD and ISSR were loaded on 1.2 percent agarose gel for visualization to detect presence or absence of the band.

Data Analysis

The amplified fragments were scored as '1' for the presence and '0' for the absence of the band generating the 0 and 1 matrix. Binary data were used to analyze using NTSYS-PC version 2.02 (Rohlf 2004). The SIMQUAL sub-program was used to calculate the Jaccard coefficient of similarity (Jaccard 1908). Similarity matrices based on these indices were calculated and used to construct the UPGMA (un-weighted pair group method with arithmetic average) dendrograms so as to elucidate the diversity among the genotypes studied (Sneath and Sokal 1973).

Table 1: List of Genotypes Used in Present Study and their Pedigree

Sr. No.	Name of Genotypes	Pedigree	Source
1.	UG-158	J 63 × TPG 41	DGR, Junagarh
2.	UG-160	GG 2 × B 95	DGR, Junagarh
3.	UG-161	GG 8 × TKG 19 A	DGR, Junagarh
4.	UG-162	GG 2× TPG 41	DGR, Junagarh
5.	UG-163	GG 20 × PBS 24030	DGR, Junagarh
6.	UG-164	ICGX 090018	ICRISAT
7.	UG-165	GG 21 × R-2001-3	DGR, Junagarh
8.	UG-167	GG 2 × TG 26	DGR, Junagarh
9.	UG-168	GG 20 × TAG 24	DGR, Junagarh
10.	UG-169	GG 20 × ICGV 86325	DGR, Junagarh
11.	UG-170	GG-7 × R-2001-3	DGR, Junagarh
12.	UG-172	TG-37 A × GG 20	DGR, Junagarh
13.	UG-173	GG 2 × ICGV 91114-1	DGR, Junagarh
14.	UG-174	TG 40 × ICGV 86325	DGR, Junagarh
15.	UG-175	PBS 24030 × TG 37 A	DGR, Junagarh
16.	UG-177	J 11 × TPG 41	DGR, Junagarh
17.	UG-178	ICGV 76 × ICGV 86305	DGR, Junagarh
18.	UG-179	ICGV 86564 × TPG 41	DGR, Junagarh
19.	UG-181	ICGV 86590 × PBS 24030	DGR, Junagarh
20.	UG-182	UG 20 × ALR-3	DGR, Junagarh
21.	UG-184	GG 5 × TPG 41	DGR, Junagarh
22.	PM -2	ICGV- 86055 × ICG- (FDRS 10)	DGR, Junagarh
23.	UG-5	Selection from ICGV-98223	DGR, Junagarh
24.	GG-7	S 206 × FEFR 81-1-9-B-B	DGR, Junagarh

RESULTS AND DISCUSSIONS

Molecular characterization is helpful in understanding the phylogenetic relationship between plant species and to reveal the genetic diversity within a given taxonomic group (Fatokun *et al.* 1993; Kaga 1996). Further, RAPD and ISSR in particular offer a simple, efficient and economic means for cultivar identification and diversity analysis (Williams *et al.* 1990).

DNA Isolation, Purification and Quantification

The amount of DNA isolated from various genotypes of *A. hypogaea* L. Ranged from 529 to 5904 mg/µl (Table 2). The genotype UG-172 yielded the highest amount of DNA (5904 ng/µl), whereas the lowest amount of DNA (599 ng/µl) was obtained from genotype UG-158. The ratio of absorbance (A260/A280) ranging from 1.70 to 1.96 revealed that the DNA obtained was free from contaminants.

Molecular Marker Analysis of RAPD and ISSR

The present study aims to analyze the extent of genetic diversity, using a total of 23 dulcimers, 20 ISSR primer, respectively, to generate DNA fingerprints of 24 *A. hypogaea* L. Genotypes with a view to detect polymorphism and access to information on diversity among these genotypes. Twenty three RAPD primers having 60% or more GC content was used for the present investigation. Out of 23 primers, 19 were amplified except P-20, OPF-17, OPK-06 and OPK-07. A total of 97 amplified bands was obtained out of which 70 were polymorphic that showed 72.16% polymorphism (Table 3). The total number of amplified bands varied between 3 (primer OPA-02) and 8 (primer OPA-10) with an average of 5 bands per primer. The overall size of PCR amplified products ranged between 100 bp to 2500 bp. The percent

polymorphism ranged from as low as 33.33 (OPA-05 and OPB-05) to as high as 100 (OPA-02, OPA-03, OPA-07, OPA-08, OPB-01, OPB-04, OPB-06 and OPC-05 (plate1-12). The average PIC was 0.216 ranging from 0.0319 to 0.449. The lowest and the highest PIC value were recorded for primer OPA-05 and OPB-07 respectively. Similar results have been reported by Talebi *et al*., (2008).

Five unique bands (a band which is present in a particular genotype, but absent in the rest of the genotypes) were detected in three genotypes *viz.,* UG174, UG167 and PM2 with 3 RAPD primers (OPA-07, OPA-08 and OPA-09). The genotypes PM2 and UG167 gave the maximum number of distinct bands *i.e.,* 2. The size of these unique bands ranged from 1500-2000 bp.

Twenty ISSR primers were used in the present investigation, out of which eighteen primers showed amplification a total of 77 amplified bands out of which 67 were found polymorphic in 24 genotypes (Table 4). The total number of amplified bands varied between 2 (UBC-813) and 6 (UBC-817, UBC873 and UBC854) with an average of 5 per primer. The polymorphism percentage ranged from as low as 20% (UBC-815) to as high as 100% in 14 primers (UBC-810, UBC-811, UBC-813, UBC-814, UBC817, UBC818, UBC-820, UBC-822, UBC-826, UBC-834, UBC836, UBC-840, UBC-845 and UBC-873 (Plate 12-24) and the average polymorphism was 87%. The overall size of PCR amplified products ranged between 100 bp to 2500 bp. PIC values ranged from 0.07 to 0.37 with an average of 0.300 across all the genotypes. One unique band was detected in PM2 genotype with UBC-817 ISSR primer. The size of this unique band was 300 bp.

Genetic Relationship and Cluster Tree analysis of RAPD and ISSR

Based on RAPD, the values of similarity coefficients ranged from 0.63 to 0.94 *i.e.,* 63-94% or genetic diversity ranged from 6 to 36%. The average similarity across all the genotypes was found to be 0.79 showing that the genotypes were moderately diverse. Based on ISSR, the values of similarity coefficients ranged from 0.54 to 0.90 *i.e.,* 54-90% or genetic diversity ranged from 10 to 46% (Table 5 and 6). The average similarity coefficient value across all the genotypes was found out to be 0.72, showing that genotypes were moderately similar.

RAPD Marker based Cluster Tree Analysis

The RAPD cluster tree analysis of 24 *A. hypogaea* L. Genotypes showed that they could be mainly divided into 4 major clusters at a similarity coefficient of 0.78 (Figure 1). Cluster I included two genotypes *viz.,* UG178 and UG184 that were related to each other at similarity coefficient of 0.80. The cluster II included 2 genotypes *viz.,* GG7 and UG179 related to each other at a similarity coefficient of 0.82.

The cluster III included 15 genotypes *viz.,* UG177, UG172, UG168, UG181, UG169, UG162, UG161, UG175, UG162, UG163, UG165, UG167, UG170, UG173 and UG174. This cluster could be divided into five sub-clusters. In sub-cluster I, containing genotypes UG172 and UG181 were related to each other at similarity coefficient of 0.88. In sub-cluster II, two genotypes UG170 and UG173 were related to each other at 0.90 similarity coefficient. In sub-cluster III, genotypes UG165 and UG169 were related to each other at 0.87 similarity coefficients while sub-cluster IV has genotypes UG162 and UG164 related to each other at 0.90 similarity coefficient value. In sub-cluster V, genotypes UG161 and UG175 were present related to each other at 0.86 similarity coefficient. The genotypes UG174, UG168, UG167, UG177, UG163 and UG162 out- grouped from sub-cluster analysis.

Cluster IV included three genotypes UG158, UG160 and UG5; UG158 and UG160 were related to each other at similarity coefficient of 0.91 and UG5 out-groped from cluster IV. The genotype UG182 was out-grouped from the cluster

analysis.

The observations for ISSR showed that they could be divided into 5 major clusters at a similarity coefficient of 0.77 (Figure 2). Cluster I included two genotypes *viz.,* UG172 and UG167found similar at a similarity coefficient of 0.78. Cluster II also included two genotypes UG5 and PM2 that were similar at similarity coefficient of 0.80. Cluster III included nine genotypes *viz.,* UG182, UG169, UG170, UG168, UG179, UG184, UG181, UG164, and UG163. It could be divided into 5 sub-clusters. In sub-cluster I, one genotype UG169 was present at 0.92 similarity coefficient. In sub-cluster II, two genotypes UG170 and UG168 were related to each other at 0.87 similarity coefficient. In sub-cluster III, genotype UG179 was present at 0.92 similarity coefficient. In sub-cluster IV, two genotypes UG184 and UG181 were related to each other at 0.90 similarity coefficient. In sub-cluster V, two genotypes UG164 and UG163 were related to each other at 0.92 similarity coefficient. Genotype UG182 out-grouped from the sub-cluster analysis.

The cluster IV included 2 genotypes, UG178 and UG173. Both the genotypes were related to each other at 0.88 similarity coefficient. The cluster V included 5 genotypes namely UG177, UG175, UG174, UG161 and UG160. Cluster V could be subdivided into two sub-groups, sub-group I including two genotypes UG175 and UG174 were related to each other at 0.91 similarity coefficient. Subgroup II, including two genotypes UG161 and UG160 were related to each other at 0.85 similarity coefficient. Genotypes UG177 was out-grouped from the sub-cluster. Also genotypes UG158 and UG165 were furthest from cluster analysis. Two and three dimensional principal component analysis based on RAPD and ISSR data (Figure 3 and 4), respectively, showed similar clustering of 24 genotypes as evident from the cluster tree analysis. PCA Based on RAPD data dice similarity coefficient value ranged from 0.82 to 0.94. Most of the genotypes tended to cluster mainly into four clusters. PCA based on ISSR data dice similarity coefficients ranged from 0.78 to 0.92, indicative of an average level of variation among the genotypes. As visible in the dendrogram, the genotypes that were closer were more similar than those that were lying apart.

Table 2: Quality and Quantity of Total Genomic DNA of *A. Hypogaea*l

S. No.	Genotype Code	Genotype	Quality (A260/A280)	Quantity (ng/µl)
1.	G_1	UG158	1.93	599
2.	G_2	UG160	1.94	2757
3.	G_3	UG161	1.93	1775
4.	G_4	UG162	1.92	3061
5.	G_5	UG163	1.90	2103
6.	G_6	UG164	1.93	1433
7.	G_7	UG165	1.88	2767
8.	G_8	UG167	1.76	4900
9.	G_9	UG168	1.87	4532
10.	G_{10}	UG169	1.87	5257
11.	G_{11}	UG 170	1.78	5064
12.	G_{12}	UG173	1.8	1620
13.	G_{13}	UG174	1.83	1571
14.	G_{14}	UG 175	1.85	3770
15.	G_{15}	UG177	1.96	907
16.	G_{16}	UG178	1.89	3483
17.	G_{17}	UG179	1.80	4250
18.	G_{18}	UG181	1.88	1863
19.	G_{19}	UG182	1.91	3627
20.	G_{20}	UG184	1.84	5250
21.	G_{21}	UG5	1.85	3501

Table 2: Contd.,				
22.	G_{22}	GG7	1.85	1953
23.	G_{23}	UG172	1.87	5904
24.	G_{24}	PM2	1.82	1469

Table 3: DNA Amplification Profile and Polymorphism Generated in *A. Hypogaea*l. Using 19 RAPD Primers

S. No.	Primer Code	Molecular Weight Range (bp)	Total No. of Bands Amplified (X)	Polymorphic Bands		PIC
				Number	Frequency (%)	
1.	OPA-02	400-1000	3	3	100.0	0.274
2.	OPA-03	300-1000	3	3	100.0	0.201
3.	OPA-05	100-1500	6	2	33.33	0.133
4.	OPA-06	300-700	3	2	66.66	0.230
5.	OPA-07	400-2000	5	5	100.0	0.172
6.	OPA-08	300-2000	7	7	100.0	0.234
7.	OPA-09	300-2000	5	2	40.0	0.031
8.	OPA-10	100-2500	8	7	87.50	0.185
9.	OPB-01	100-1000	6	6	100.0	0.153
10.	OPB-02	200-1500	7	5	71.42	0.288
11.	OPB-03	300-1500	4	2	50.0	0.210
12.	OPB-04	200-1500	6	6	100.0	0.328
13.	OPB-05	400-2000	6	2	33.33	0.142
14.	OPB-06	200-600	3	3	100.0	0.284
15.	OPB-07	300-1000	4	0	0.0	0.449
16.	OPC-05	300-800	4	4	100.0	0.149
17	OPD-02	200-1000	7	5	71.42	0.290
18	C-19	100-1000	5	4	80.0	0.168
19	S-35	100-1000	5	2	40.0	0.206
	Total		97	**70**	**72.16**	**0.216**

Table 4: DNA Amplification Profile and Polymorphism Generated in *A. Hypogaea* L. using 18 ISSR Primers

S. No.	Primer Code	Ta* (^{o}C)	Molecular Weight Range (bp)	Total no. of Bands Amplified	Polymorphic Bands		PIC
					Number	Frequency (%)	
1.	UBC-810	42.9	400-900	4	4	100.0	0.218
2.	UBC-811	43.3	500-900	3	3	100.0	0.239
3.	UBC-813	43.3	500-1000	2	2	100.0	0.31
4.	UBC-814	41.3	300-1500	4	4	100.0	0.392
5.	UBC-815	44.9	200-1000	5	1	20.0	0.079
6.	UBC-817	52	300-2000	4	4	100.0	0.353
7.	UBC-818	52	400-900	4	4	100.0	0.328
8.	UBC-820	50	100-1000	5	5	100.0	0.369
9.	UBC-822	45	300-1000	4	2	50.00	0.076
10.	UBC-824	43.3	100-2000	5	4	80.0	0.392
11.	UBC-826	52	300-800	6	6	100.0	0.462
12.	UBC-834	49.9	400-1500	4	4	100.0	0.379

Table 4: Contd.,							
13.	UBC-836	43.3	500-1500	4	4	100.0	0.228
14.	UBC-840	45	200-900	4	4	100.0	0.178
15.	UBC-845	47.7	500-2500	3	3	100.0	0.229
16.	UBC-848	55.5	200-600	4	3	70.62	0.163
17.	UBC-854	48.0	400-2500	6	4	66.6	0.255
18.	UBC-873	45	400-1000	6	6	100.0	0.348
	Total			**77**	**67**	**87.00**	**0.300**

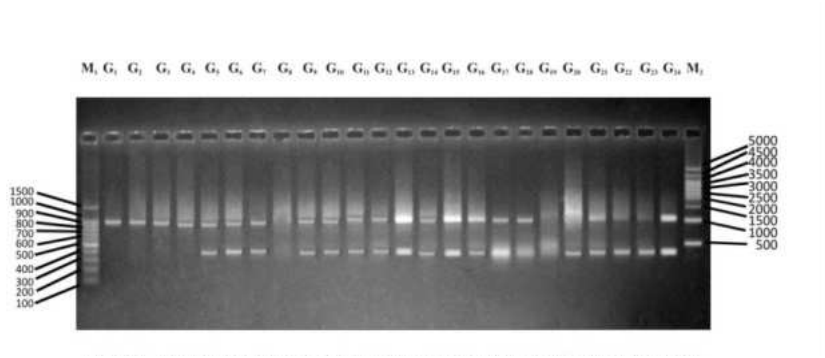


Plate 1: RAPD Profile Generated through OPA-02{5' TGCCGAGCTG3'}

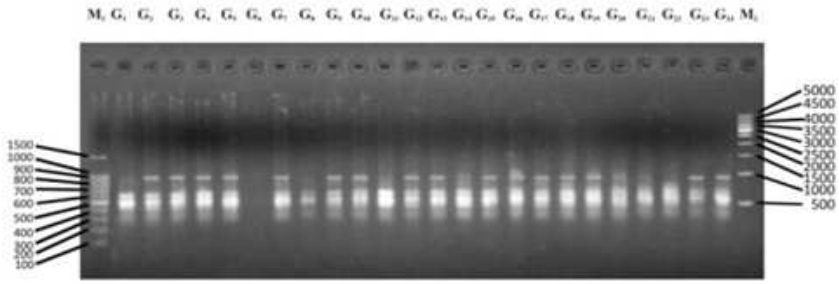
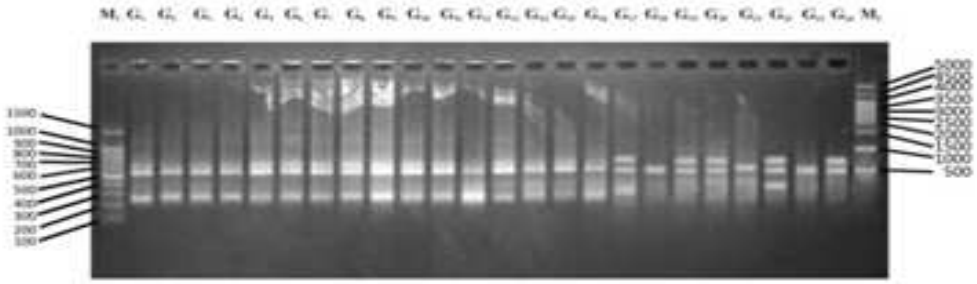


Plate 2: RAPD Profile Generated through OPA-03 {5' AGTCAGCCAC3'}



M1 – 100 bp DNA Ladder **M2** – 500 bp DNA Ladder

G_1-G_{24} represent following *Arachis hypogaea* L. genotypes

G_1 –UG158	G_2 -UG160	G_3 -UG161	G_4 -UG162	G_5 -UG163	G_6 -UG164
G_7 –UG165	G_8 -UG167	G_9 -UG168	G_{10} -UG169	G_{11} -UG170	G_{12} -UG173
G_{13} –UG174	G_{14} -UG175	G_{15} -UG177	G_{16} -UG178	G_{17} -UG179	G_{18} -UG181
G_{19} –UG182	G_{20} -UG184	G_{21} -UG5	G_{22} -GG7	G_{23} -UG172	G_{24} -PM2

Plate 3: RAPD Profile Generated through OPA-06 {5' GGPCCCTGAC3'}

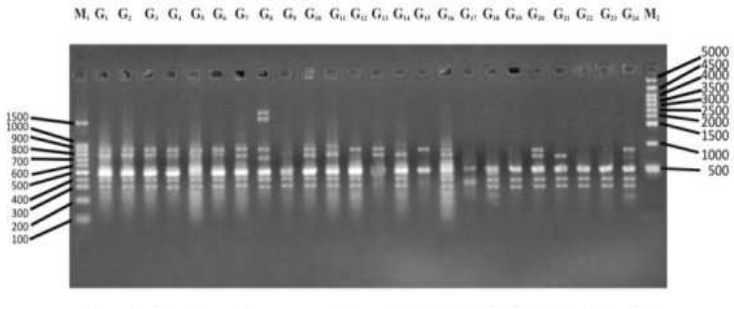


Plate 4: RAPD Profile Generated through OPA-08 {5' GTGACGTAGG3'}

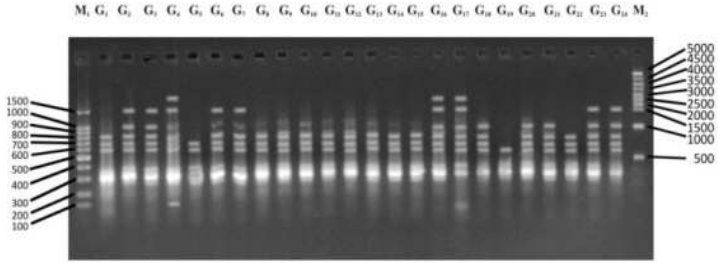
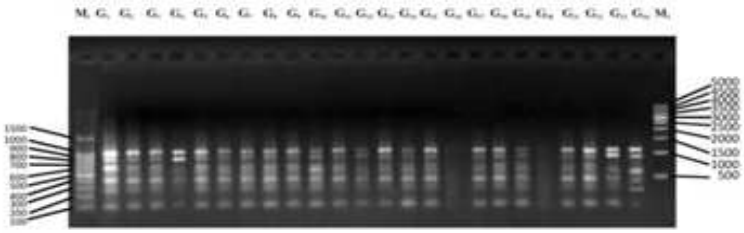


Plate 5: RAPD Profile Generated through OPA-10 {5' GTGATCGCAG3'}



M1 = 100 bp DNA Ladder M2 = 500 bp DNA Ladder

G_1-G_{24} represent following *Arachis hypogaea* L. genotypes

G_1 –UG158	G_2 -UG160	G_3 -UG161	G_4 -UG162	G_5 -UG163	G_6 -UG164
G_7 –UG165	G_8 -UG167	G_9 -UG168	G_{10} -UG169	G_{11} -UG170	G_{12} -UG173
G_{13} –UG174	G_{14} -UG175	G_{15} -UG177	G_{16} -UG178	G_{17} -UG179	G_{18} -UG181
G_{19} –UG182	G_{20} -UG184	G_{21} -UG5	G_{22} -GG7	G_{23} -UG172	G_{24} -PM2

Plate 6: RAPD Profile Generated through OPB-01 {5' GTTTCGCTCC3'}

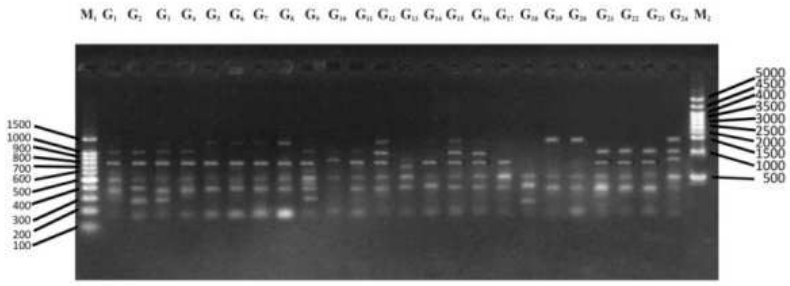


Plate 7: RAPD Profile Generated through OPB-02 {5' TGATCCCGG3'}

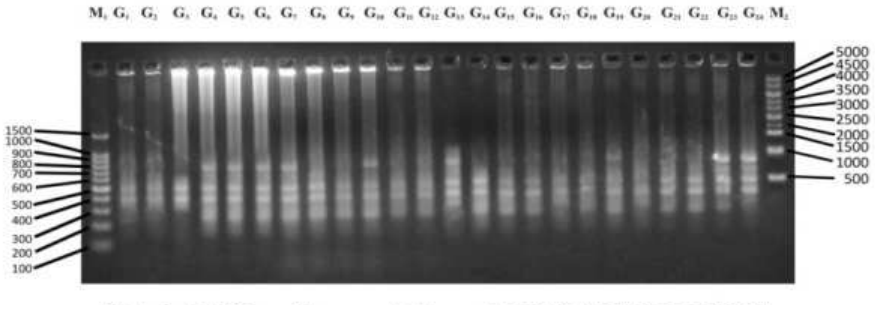
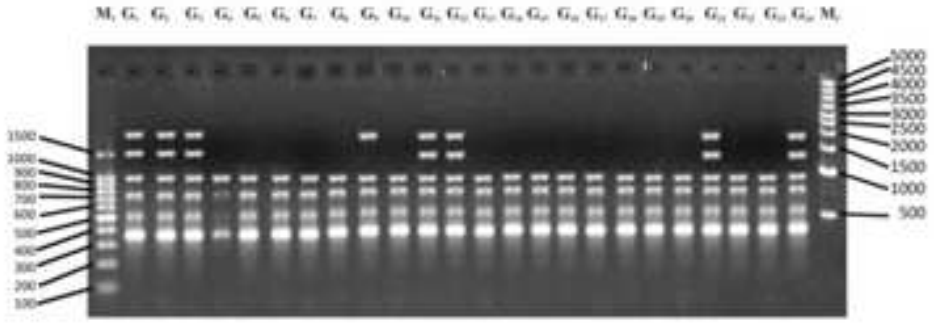


Plate 8: RAPD Profile Generated through OPB-03 {5' CATCCCCTG3'}



M1 = 100 bp DNA Ladder **M2** = 500 bp DNA Ladder

G_1-G_{24} represent following *Arachis hypogaea* L. genotypes

G_1 –UG158	G_2 -UG160	G_3 -UG161	G_4 -UG162	G_5 -UG163	G_6 -UG164
G_7 –UG165	G_8 -UG167	G_9 -UG168	G_{10} -UG169	G_{11} -UG170	G_{12} -UG173
G_{13} –UG174	G_{14} -UG175	G_{15} -UG177	G_{16} -UG178	G_{17} -UG179	G_{18} -UG181
G_{19} –UG182	G_{20} -UG184	G_{21} -UG5	G_{22} -GG7	G_{23} -UG172	G_{24} -PM2

Plate 9: RAPD Profile Generated through OPB-05 {5'TGCGCCCTTC3'}

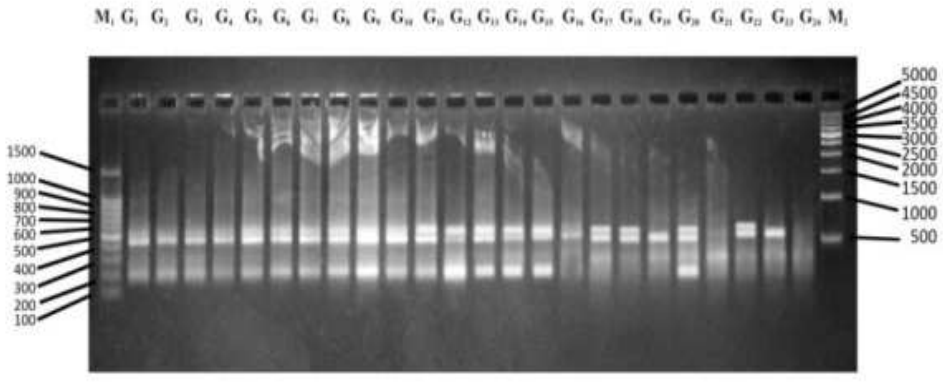


Plate 10: RAPD Profile Generated through OPB-06 {5'TGCTCTGCCC3'}

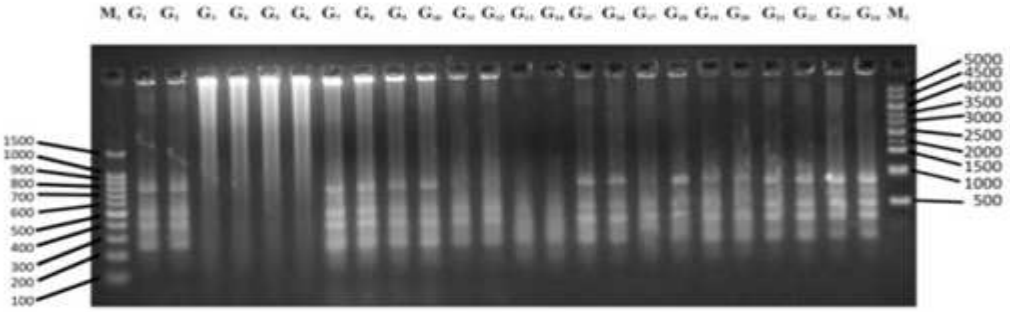
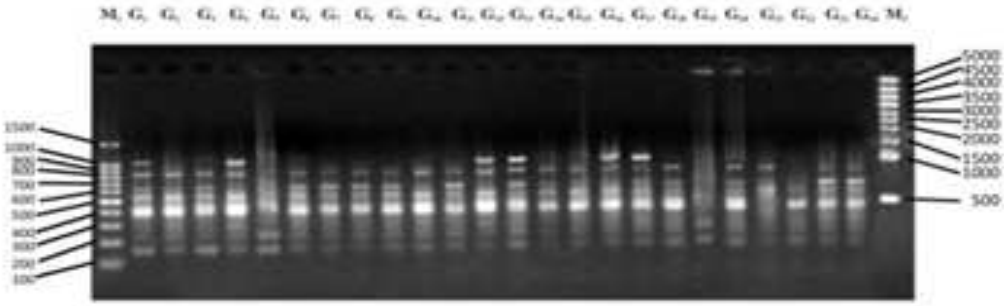


Plate 11: RAPD Profile Generated through OPC-05 {5'GATGACCGCC3'}



M1 = 100 bp DNA Ladder M2 = 500 bp DNA Ladder

G_1-G_{24} represent following *Arachis hypogaea* L. genotypes

G_1 –UG158	G_2 -UG160	G_3 -UG161	G_4 -UG162	G_5 -UG163	G_6 -UG164
G_7 –UG165	G_8 -UG167	G_9 -UG168	G_{10} -UG169	G_{11} -UG170	G_{12} -UG173
G_{13} –UG174	G_{14} -UG175	G_{15} -UG177	G_{16} -UG178	G_{17} -UG179	G_{18} -UG181
G_{19} –UG182	G_{20} -UG184	G_{21} -UG5	G_{22} -GG7	G_{23} -UG172	G_{24} -PM2

Plate 12: RAPD Profile Generated through OPD-02 {5'GGACCCAACC3'}

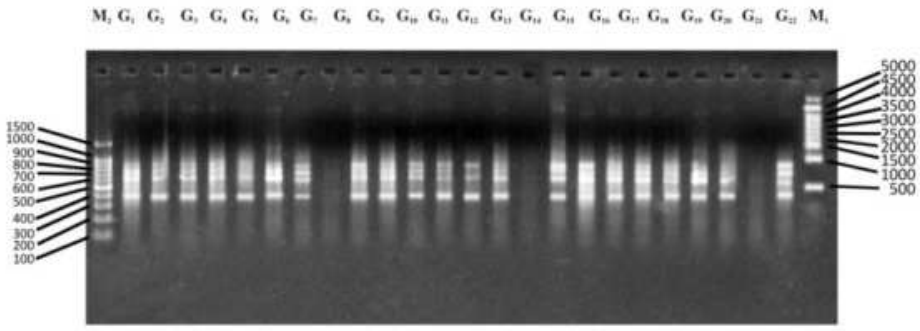


Plate 13: ISSR Profile Generated through UBC-810 {5'(GA),T3'}

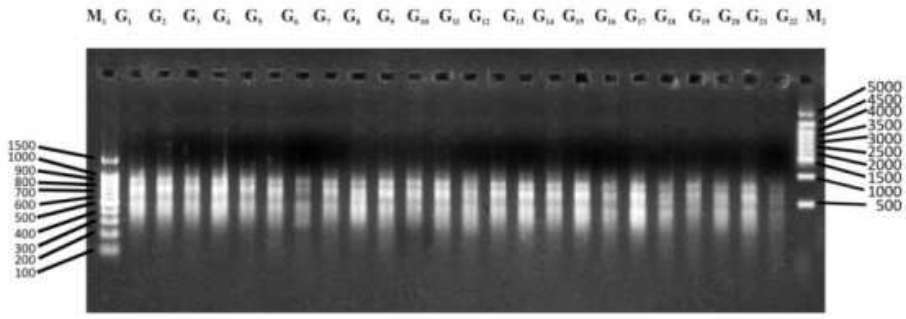
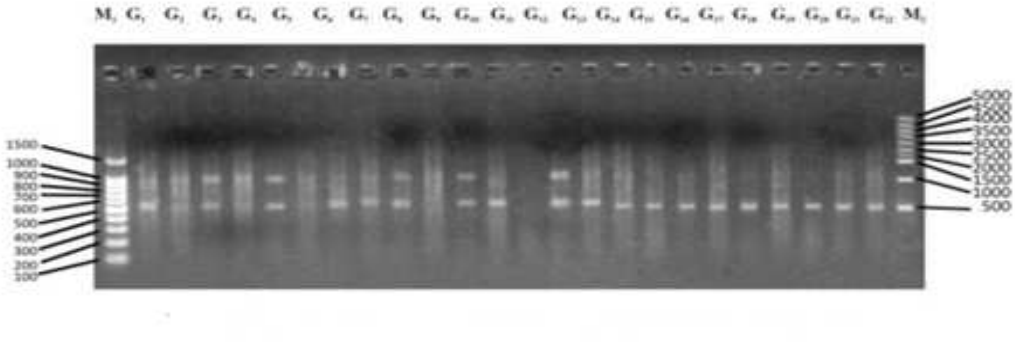


Plate 14: ISSR Profile Generated through UBC-811{5'(GA),C3'}



M1 = 100 bp DNA Ladder **M2** = 500 bp DNA Ladder

G_1-G_{24} represent following *Arachis hypogaea* L. genotypes

G_1 –UG158	G_2 -UG160	G_3 -UG161	G_4 -UG162	G_5 -UG163	G_6 -UG164
G_7 –UG165	G_8 -UG167	G_9 -UG168	G_{10} -UG169	G_{11} -UG170	G_{12} -UG173
G_{13}–UG174	G_{14}-UG175	G_{15}-UG177	G_{16} -UG178	G_{17} -UG179	G_{18}-UG181
G_{19}–UG182	G_{20} -UG184	G_{21}-UG5	G_{22} -GG7	G_{23} -UG172	G_{24} -PM2

Plate 15: ISSR Profile Generated through UBC-813 {5'(CT),T3}

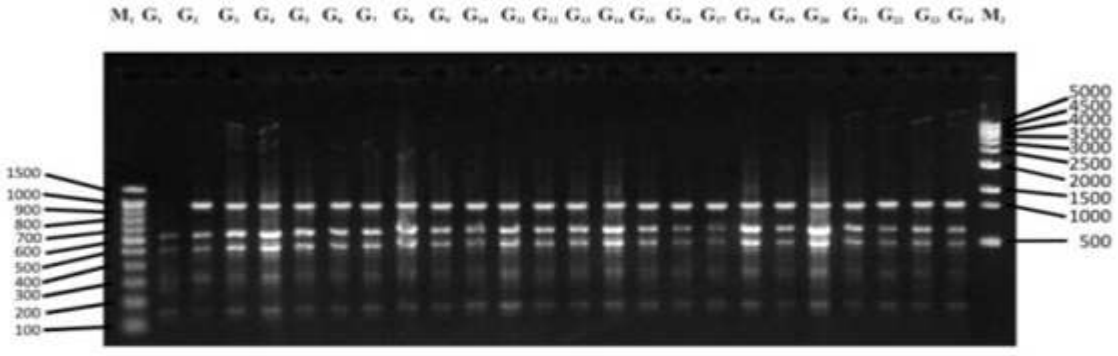


Plate 16: ISSR Profile Generated through UBC-815 {5'(CT),G3'}

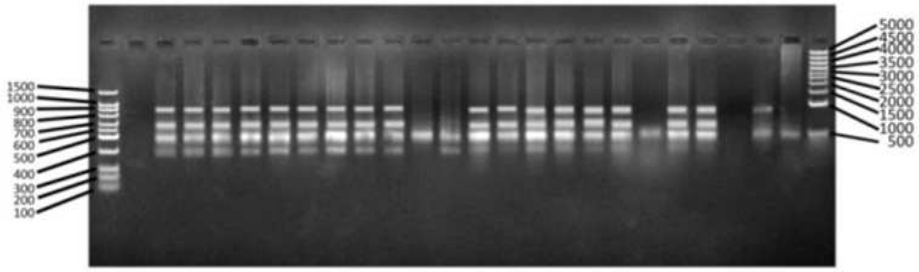
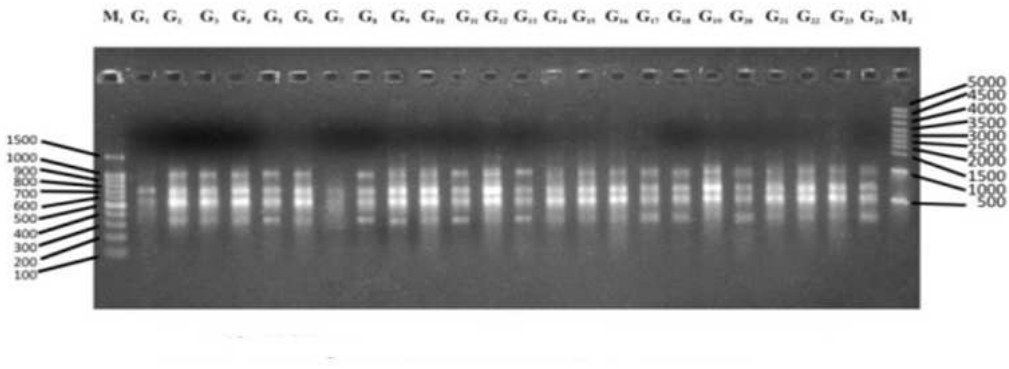


Plate 17: ISSR Profile Generated through UBC-818 {5'(CA),A3'}



M1 = 100 bp DNA Ladder **M2** = 500 bp DNA Ladder

G_1-G_{24} represent following *Arachis hypogaea* L. genotypes

G_1 –UG158	G_2 -UG160	G_3 -UG161	G_4 -UG162	G_5 -UG163	G_6 -UG164
G_7 –UG165	G_8 -UG167	G_9 -UG168	G_{10} -UG169	G_{11} -UG170	G_{12} -UG173
G_{13}–UG174	G_{14}-UG175	G_{15}-UG177	G_{16} -UG178	G_{17} -UG179	G_{18}-UG181
G_{19}–UG182	G_{20} -UG184	G_{21}-UG5	G_{22} -GG7	G_{23} -UG172	G_{24} -PM2

Plate 18: ISSR Profile Generated through UBC-822 {5'(TC),A3'}

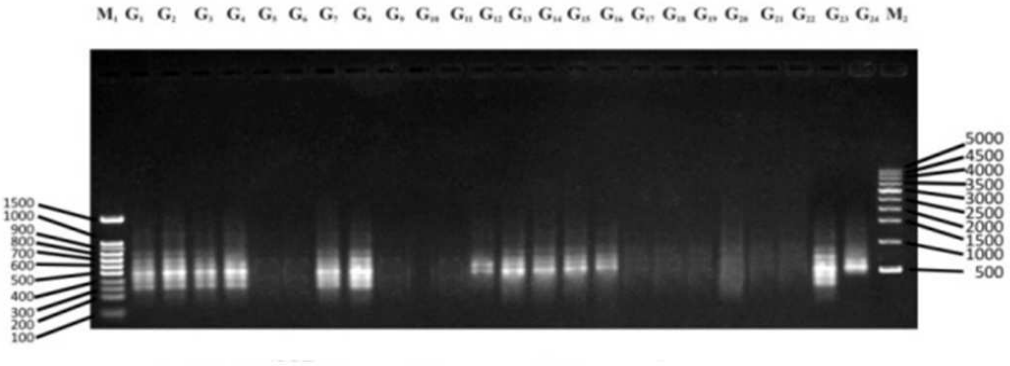


Plate 19: ISSR Profile Generated through UBC-826 {5'(AC),C3'}

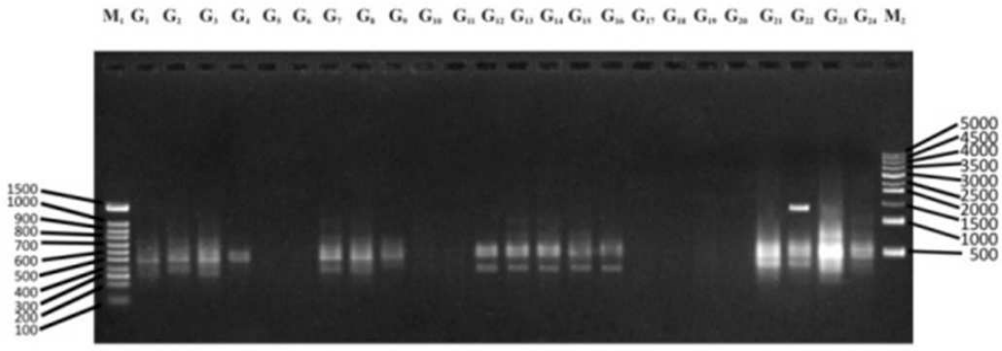
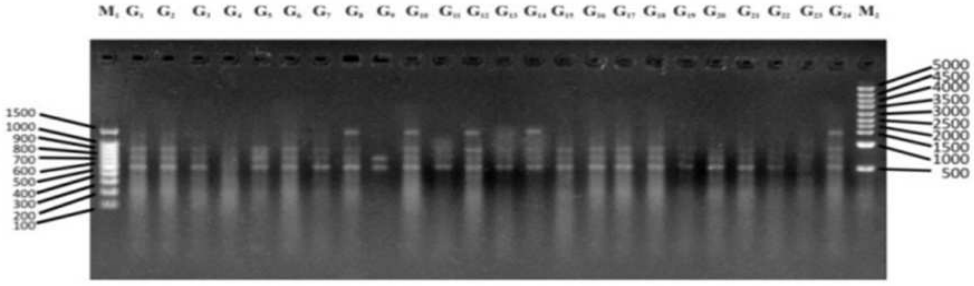


Plate 20: ISSR Profile Generated through UBC-834 {5'(AC),Y*T3'}



M1 = 100 bp DNA Ladder **M2** = 500 bp DNA Ladder

G_1-G_{24} represent following *Arachis hypogaea* L. genotypes

G_1 –UG158	G_2 -UG160	G_3 -UG161	G_4 -UG162	G_5 -UG163	G_6 -UG164
G_7 –UG165	G_8 -UG167	G_9 -UG168	G_{10} -UG169	G_{11} -UG170	G_{12} -UG173
G_{13}–UG174	G_{14}-UG175	G_{15}-UG177	G_{16} -UG178	G_{17} -UG179	G_{18}-UG181
G_{19}–UG182	G_{20} -UG184	G_{21}-UG5	G_{22} -GG7	G_{23} -UG172	G_{24} -PM2

Plate 21: ISSR Profile Generated through UBC-836 {5'(AG),YA3'}

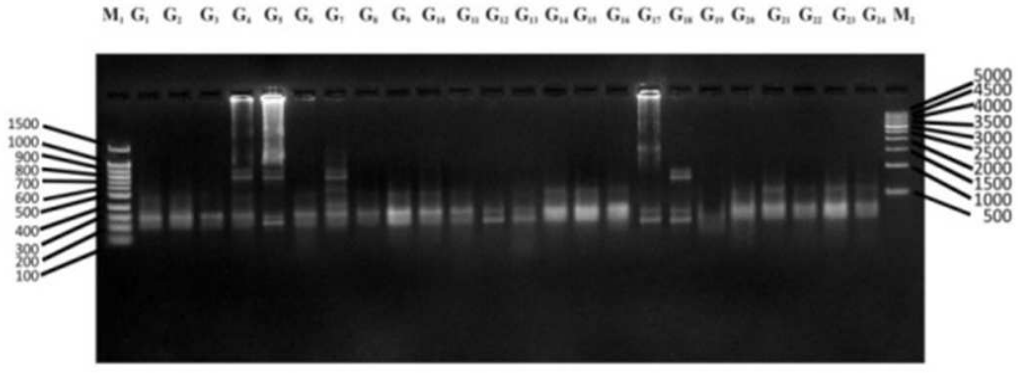


Plate 22: ISSR Profile Generated through UBC-840 {5'(GA),YT3'}

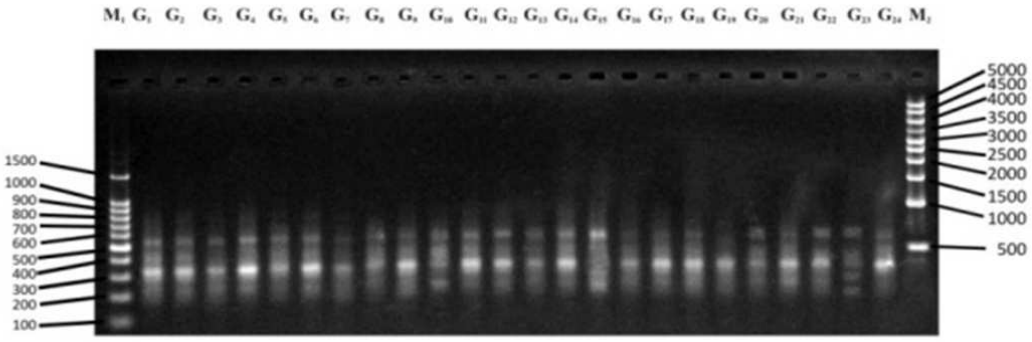
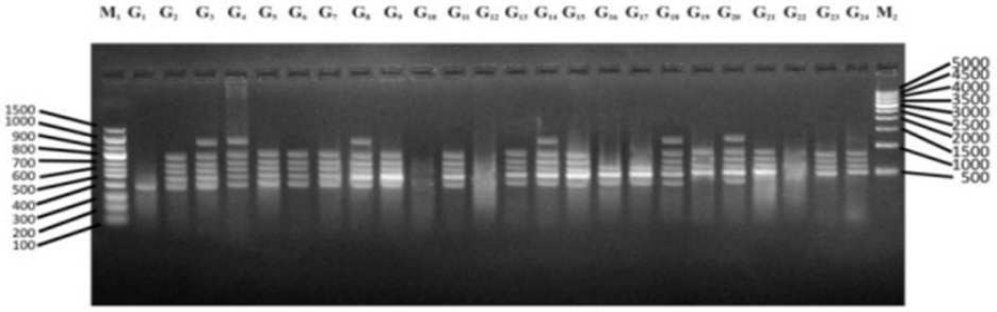


Plate 23: ISSR Profile Generated through UBC-848 {5'(CA),RG3'}



M1 = 100 bp DNA Ladder **M2** = 500 bp DNA Ladder

G_1-G_{24} represent following *Arachis hypogaea* L. genotypes

G_1 –UG158	G_2 -UG160	G_3 -UG161	G_4 -UG162	G_5 -UG163	G_6 -UG164
G_7 –UG165	G_8 -UG167	G_9 -UG168	G_{10} -UG169	G_{11} -UG170	G_{12} -UG173
G_{13}–UG174	G_{14} -UG175	G_{15}-UG177	G_{16} -UG178	G_{17} -UG179	G_{18}-UG181
G_{19}–UG182	G_{20} -UG184	G_{21}-UG5	G_{22} -GG7	G_{23} -UG172	G_{24} -PM2

Plate 24: ISSR Profile Generated through UBC-873 {5'(GATA),3'}

CONCLUSIONS

Evaluation of genetic diversity based on morphological features has not proven to be efficient as they are highly influenced by environments. To overcome these problems, biochemical and molecular techniques have been found to yield better results. RAPD and ISSR marker technique is a PCR based method, which involves amplification of DNA present at an amplifiable distance in between two identical microsatellite repeats oriented in opposite directions. Such DNA markers are considered a best tool for determining genetic relationship/diversity as they are abundant in number, highly polymorphic and is independent of environmental interaction *i.e.* are highly heritable. The development of precision molecular techniques for genetic analysis has almost replaced the morphological and biochemical analysis to a great extent. In the present study RAPD and ISSR banding pattern in plates differ significantly showed genotypes are diverse as well as a result of dendrogram showed that similar or related genotypes fall in the same cluster in both RAPD and ISSR.

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